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Mycotoxins: Occurrence, Chemistry, Biological Activity[1]

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Mycotoxins are secondary fungal metabolites capable of eliciting a toxic response, a mycotoxicosis, in a living host; ergotism and mushroom poisoning are among the earliest recognized examples. Unfortunately, the broad implications of the problem were not recognized until World War II when, in Russia, humans eating moldy over-wintered grain suffered severe dermal necroses, hemorrhages, leukopenia, and bone marrow destruction; mortality rates were severe, as high as 60% (1). About the same period, large numbers of horses vital to the Russian economy and army transport system suffered similar symptoms from eating hay molded with *Stachybotrys alternans* var. *jateli* (2). A fascinating, almost Machiavelian, story of this latter outbreak from a politician's viewpoint is woven in Nikita Krushchev's memoirs (3). Yet full scientific recognition was not given to the mycotoxin problem until it was discovered that the aflatoxins, which were responsible for the deaths of a large number of turkey poults in England in 1960, were extremely potent carcinogens in laboratory animals (4).

Various surveys have revealed that the mycotoxicoses are not restricted to any geographical or climatic regions. However, the scope and magnitude of the problem are difficult to define—only a relatively few fungi and the toxins they produce have been definitively implicated in a mycotoxicosis. In most cases, evidence is circumstantial for a number of reasons: (*i*) mycotoxins often occur in very low concentrations and may be difficult to detect; (*ii*) the suspect food or feed has often been disposed of by the time a mycotoxicosis is indicated and is not longer available for analyses; (*iii*) host symptoms are often ill-defined or nonspecific, *e.g.*, anorexia, reduced weight gains and lower feed conversion efficiency in livestock; and (*iv*) veterinarians and physicians are not trained or sufficiently familiar with symptomatology even in acute cases. A further confusing element has been the discovery of many toxic mold metabolites to which no disease has been attributed (penicillic acid, rubratoxin, "yellow rice" toxins) and, conversely, there are diseases suspected of being mycotoxicoses for which no toxin has yet been found (sheep lupinois, fescue foot, Balkan nephrosis syndrome, paspalum staggers, leukoencephalomalacia).

Those compounds that can be implicated with some certainty in a mycotoxicosis are shown in table 1; somewhat less certain are those listed in table 2. A number of the toxins listed in the two tables have been shown to be carcinogenic for various laboratory animals, but circumstantial evidence for human involvement has only been reported for the aflatoxins (table 3).

Cereal grains, peanuts, and cottonseed appear to be the most important food and feed substances that may be contaminated with mycotoxins (5). However, probably no edible substance can be regarded as absolutely safe from possible mycotoxin contamination. Mycotoxin production can occur in the field, during harvest, processing, storage, and shipment of a given commodity. Factors governing production of various mycotoxins are not completely understood although moisture and temperature probably play the most important roles. Numerous reviews on all aspects of the mycotoxin problem have been published (6–11).

Biological effects are as varied as the toxins themselves and, unfortunately,

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[2]Agricultural Research Service, U.S. Department of Agriculture.

TABLE 1. *Compounds involved in mycotoxicoses.*

Toxin	Producing fungi	Susceptible host	Biological effects	Ref.
Aflatoxin	*Aspergillus flavus, A. parasiticus*	Mammals, fish, birds	Hepatotoxin, cancer	5
Penitrem A	*Penicillium palitans, P. crustosum*	Cattle, horse, sheep	Tremorgenic, convulsant	60,61
T-2	*Fusarium tricinctum*	Cattle, man?	Dermal necrosis, hemorrhage	56
F-2	*Gibberella zeae*	Swine	Vulvovaginitis, abortion	74
Slaframine	*Rhizoctonia leguminicola*	Cattle	Excess salivation	9
Sporidesmins	*Pithomyces chartarum*	Cattle, sheep	Hepatotoxin, facial eczema	9
Ochratoxin A	*P. viridicatum, A. ochraceous*	Swine, man?	Nephrotoxin	8
Psoralens	*Sclerotinia sclerotiorum*	Man	Dermotoxin	10
Citrinin	*P. viridicatum P. citrinum*	Swine	Nephrotoxin	8
Vomitoxin	*F. graminearum*	Swine, man?	Vomiting	58
Maltoryzine	*A. oryzae*	Cattle	Death	79
Unidentified	*Phomopsis leptostromiformis*	Sheep	Hepatotoxin	80
Diplodiatoxin	*Diplodia maydis*	Cattle, sheep	Nephritis, mucoenteritis	81

because of diagnostic difficulties usually only the more acute and dramatic manifestations are observed. Probably the most important aspect involves exposure of a host to subacute doses; *e.g.*, livestock exhibit poor weight gains and lowered feed efficiency; humans may contract hepatomas and suffer from degeneration of the hematopoietic system. The broad scope of the mycotoxin problem may

TABLE 2. *Compounds suspected of being mycotoxins.*

Toxin	Producing fungi	Possible host	Biological effects	Ref.
Sterigmatocystin	*Aspergillus flavus*	Mammals	Carcinogenic	8
Yellow rice toxins				
Luteoskyrin	*Penicillium islandicum*	Man	Hepatotoxin	
Cyclochlorotine	*P. islandicum*	Man	Hepatotoxin	
Citreoviridin	*P. citreoviride*	Man	Neurotoxin	
Rugulosin	*P. rugulosum*	Man	Carcinogen	
Rubratoxin	*P. rubrum*	Cattle	Hepatotoxin	8
Fusaranon-X	*Fusarium nivale*	Man, swine	Vomiting	82
Nivalenol	*F. nivale*	Man, swine	Vomiting	83
Cytochalasin E	*A. glavatus*	Man	Death	84
PR toxin	*P. roqueforti*	Cattle	Abortion	85
Patulin	*P. urticae*	Cattle	Death	8

be best illustrated in this review by four families of these substances: aflatoxins, trichothecenes, tremorgens, and F-2 toxin (zearalenone).

AFLATOXINS

The aflatoxins, a family of closely related substances produced by *Aspergillus flavus* and *A. parasiticus,* have been studied the most intensively. There are currently 13 of these compounds which have been shown to occur in

TABLE 3. *Mycotoxin carcinogens.*[a]

Mycotoxin	Detected naturally	Target tissue	Regular dose (ppm, oral)[b]	Type of lesion
Aflatoxin B_1	+	Liver, kidney, trachea subcutaneous tissue	0.5–1.5, rat	Hepatoma, subcutaneous sarcoma
Aflatoxin G_1	+	Liver, kidney, glandular, stomach, sucutaneous tissue	1–3, rat	
Sterigmatocystin	+	Liver, subcutaneous tissue	30–100, rat	Hepatoma
Luteoskyrin		Liver	50–100, mouse	Hepatoma
Cyclochlorotine		Liver		Hepatoma
Patulin	+	Subcutaneous tissue		Subcutaneous sarcoma
Penicillic acid	+	Subcutaneous tissue		Subcutaneous sarcoma
Rugulosin		Liver	200, mouse	Hepatoma
Griseofulvin		Liver	5,000–10,000, mouse	Hepatoma

[a]Table is adapted from Enomoto and Saito (ref. 86).
[b]Oral dosage per day.

nature; all possess a coumarin nucleus fused to a bifuran moiety and contain in addition either a pentenone ring (B series) or a 6-membered lactone (G series) (fig. 1).

Aflatoxins appear to constitute a contamination problem primarily in peanuts and peatnut products, cottonseed meal, and in some cereal grains; however, many other foods and feeds have also been reported to be contaminated. The occurrence of aflatoxin is usually associated with poor storage conditions, although a growing body of evidence indicates that these compounds may also be produced in the field, *i.e.*, on the developing plant or, more correctly, on the fruit of that plant. If this is the case, then the scientific, technological, and legal implications of the aflatoxin problem will become even more complex.

Toxicologically, aflatoxin may be regarded as a quadruple threat; it can function as a potent toxin, a carcinogen, a teratogen, and a mutagen. The LD_{50} of aflatoxin B_1 to various species is shown in table 4.

There is, of course, no established toxic dose for humans; but strong circumstantial evidence from Southeast Asia (12), India (13), and Africa (14), plus a suspect case in Germany (15), indicates that aflatoxins have been involved in human deaths, particularly among children. The response of macaque monkeys to acute toxic doses of aflatoxin B_1 is strikingly similar to an acute children's disease, Reye's Syndrome, in Thailand children (16). Overt symptoms in both monkeys and children include fever, vomiting, diarrhea, coma, and convulsions. Histopathology reveals fatty degeneration of the liver, heart, and kidneys; marked cerebral edema with neural degeneration; and lymphocytolysis. Examination of foods consumed by the Thai show a considerable degree of aflatoxin contamination (17, 18). Chemical analyses on autopsied Thai children by Shank and his colleagues (12) revealed aflatoxin in 22 out of 23 cases, particularly in the liver.

Toxic effects in domestic animals are shown in table 5. Liver damage is the usual symptom and, undoubtedly, at subacute doses feed efficiency and growth rate are affected; this results in economic losses to the farmer. It has been demonstrated that in poultry there is an increased fragility of the capillaries

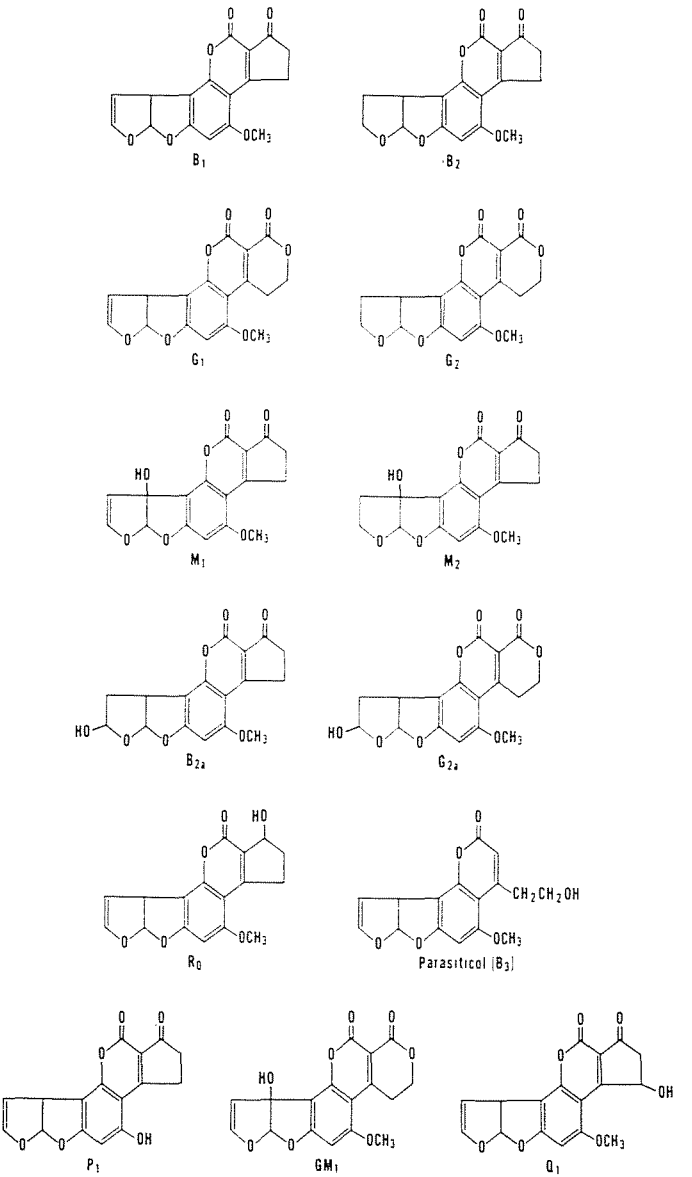


FIG. 1. *Structures of the naturally occurring aflatoxins.*

which results in bruising of the birds during mechanical processing (19). This along causes a $6 million loss to processors.

Aflatoxin has been demonstrated to be a hepatocarcinogen in various laboratory animals including ducks, rainbow trout, ferrets, rats, and mice. Aflatoxin carcinogenesis recently has been reviewed in depth by Wogan (20). Trout appear to be the most sensitive host: doses as low as 0.8 ppb in the diet caused hepatomas after 2 years, and a dose as low as 0.4 ppb caused hepatomas in trout after a total consumption of only 0.06 μg aflatoxin B_1. In addition to hepa-

TABLE 4. *Aflatoxin B_1 single dose* (per os) *LD_{50} value in various species.*

Species	LD_{50} mg/kg body wt.
Duckling	0.3–0.6
Trout	0.8
Dog	1.0
Guinea pig	1.4–2.0
Monkey	2.2
Mouse	9.0
Rat	5.5–17.9
Pig (6–7 kg)	0.6
Sheep	2.0
Chicken	6.3

tomas, aflatoxin has also been implicated in neoplasm induction in the glandular stomach, kidney, lung, salivary and lachrymal glands, the colon, and skin. Recently it has been shown at our laboratory that aflatoxin B_1 functioned as a tumor-initiating substance but had no promoter activity.

In man, evidence for liver cancer induction by aflatoxin is, of necessity, only circumstantial; epidemiological data implicating aflatoxin in carcinogenesis have been gathered in Africa, India, and Southeast Asia (12, 13, 17, 21–26). In most cases evidence is based on a relatively high incidence of hepatomas in geographical areas where moldy, aflatoxin-contaminated food is consumed.

Aflatoxin has been shown to be teratogenic in the chick embryo (27) and in the hamster (28) In the chick embryo some of the effects commonly observed include decreased growth, exencephaly, anopthalmia, microopthalmia, cleft pallet, and malformation of the maxilla. No effects have been described for humans.

TABLE 5. *Dietary aflatoxin concentrations causing toxicosis.*

Species	Age	Aflatoxin content (ppm)	Duration of feeding	Effects
Calves	Weanling	0.2–2.2	16 weeks	Stunting, death, liver damage
Steers	2 years	0.2–0.7	20 weeks	Liver damage
Cows	2 years	2.4	7 months	Liver damage
Pigs	Newborn	0.23	4 days	Stunting
Pigs	2 weeks	0.17	23 days	Anorexia, stunting, jaundice
Pigs	4–6 weeks	0.4–0.7	3–6 months	Stunting, liver damage
Chickens	1+week	0.8	10 weeks	Stunting, liver damage
Ducks	Unknown	0.3	6 weeks	Liver damage, death

Studies related to mutagenicity revealed that aflatoxin induced chromosome aberrations in seedling roots of *Vicia faba* (29), in a rat kangaroo cell line (30), and in human leukocytes (31); induced dominant lethal mutations in mice (32); and was mutagenic in *Neurospora crassa* causing multilocus deletions (33). Upon activation by rat liver homogenates, aflatoxin B_1 was converted to a potent frameshift mutagen for *Salmonella typhimurium* (34).

Evidence exists that the toxicity and carcinogenicity of aflatoxin B_1 may result from conversion of the compound to a more reactive intermediate. The metabolism of aflatoxin B_1 by various mammals to the fluorescent hydroxylated

metabolite aflatoxin M_1 has been demonstrated, but usually only small amounts of the total toxins administered have been recovered as identifiable fluorescent compounds from feces, urine, and milk. From human urine only about 5% is recovered as M_1 (35). These low excretions suggested the existence of a major pathway for the *in vivo* formation of nonfluorescent metabolites. Studies at the Massachusetts Institute of Technology and at the University of California (Davis) using labeled aflatoxin reveal that a higher proportion, about 20% of the toxin administered, is excreted in urine as water-soluble, nonfluorescent glucuronides and sulphates (36). Most of the administered toxin appear to be metabolized by the liver with the enzymes involved being located primarily in the endoplasmic reticulum or microsomes. As a result of a series of investigations primarily by Patterson, Roberts, and Allcroft in England, a tentative scheme (fig. 2) has been advanced for the *in vivo* metabolism of aflatoxin (37).

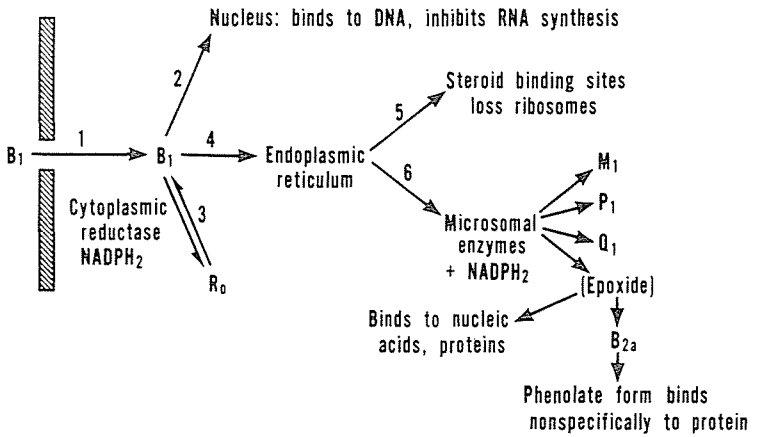


FIG. 2. *Aflatoxin B_1 metabolism.* (ref. 37).

The formation of aflatoxicol as noted in fig. 2 is rapidly affected by a soluble enzyme preparation obtained from avian and rabbit liver macerates centrifuged at 105,000 x g (38). However, when aflatoxicol is incubated *in vitro* with both the 105,000 x g supernatant and a washed microsomal preparation, the hemiacetal of aflatoxin B_1 is formed instead of the theoretical hemiacetal of aflatoxicol (39). At the same time, traces of aflatoxin B_1 are detected; this suggests that aflatoxicol had first been oxidized to B_1 and then hydrated to form the hemiacetal, B_{2a}. Whether this reverse reaction occurs *in vivo* is uncertain, for if a high $NADPH_2$:NADP ratio is maintained in the birds it would appear to favor the formation of aflatoxicol. However, the proportion of aflatoxin so metabolized may depend, among other things, on the partitioning of the toxin between "active sites" on the endoplasmic reticulum and sites on the soluble enzyme proteins.

The microsomal fraction from avian livers rapidly converts B_1 to the relatively nontoxic B_{2a} (40). However, B_{2a} at physiological pH (7.4) is highly unstable, probably as a result of its existence in phenolate form (41).

The phenolate form reacts rapidly with proteins or amino acids probably as a result of Schiff base formation. The product is unstable and degrades, possibly by autooxidation, to give yellow products (39).

Since B_{2a} is relatively nontoxic and yet young birds and guinea pigs are highly susceptible to aflatoxin, it is possible that a very toxic, short-lived epoxide may result initially from enzymic attack on the vinyl ether double bond in the

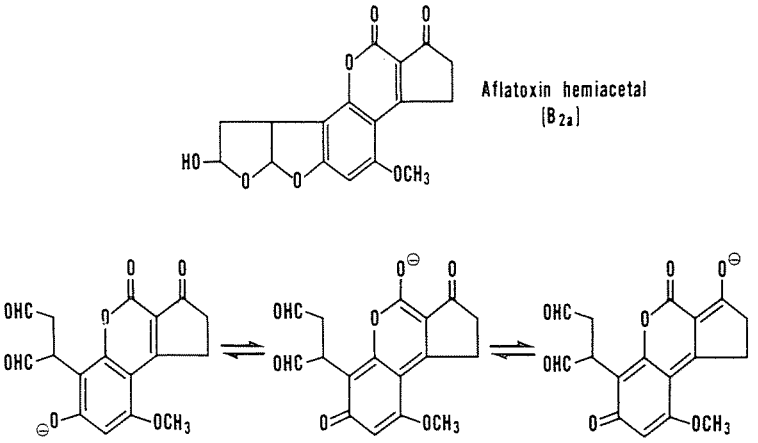


FIG. 3. *Resonance forms of the phenolate ion of aflatoxin* B_{2a}. (ref. 41).

aflatoxin molecule (42). The formation of a reactive intermediate would thus fit aflatoxin in with most, if not all, of the other known nonalkylating carcinogens that actually exist as precarcinogens and require conversion by the host into the carcinogenic and reactive structures. The investigations of Patterson and Allcroft (43) on metabolism of aflatoxin in various animal species suggested that a short-lived epoxide, possibly formed during the conversion of B_1 to B_{2a}, might

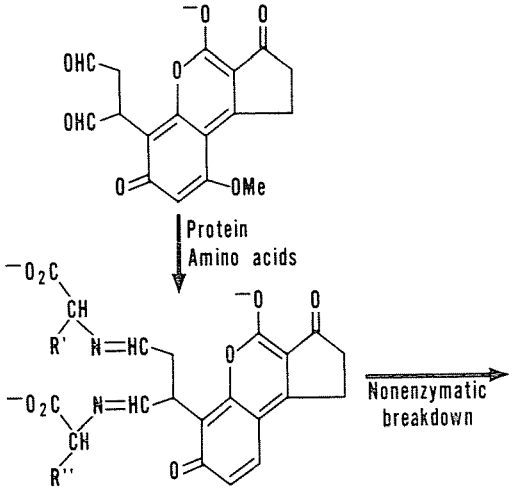


FIG. 4. *Reaction of aflatoxin* B_{2a} *in phenolate form to form Schiff base.* (ref. 39).

be this intermediate. Epoxidation of the K-region in carcinogenic polycyclic aromatic hydrocarbons has also been suggested to be the first step in their metabolism (44). The K-region is a site that is particularly electron rich and, therefore, very reactive. Among polycyclic hydrocarbons this site appears to be the key to potential carcinogenicity. Experimental evidence that naphthalene epoxide is formed *in vitro* by rabbit microsomal preparations supports this view. Of particular significance was the discovery of the intramolecular migration of ring substituents during the course of aromatic oxidation (the so-called

NIH shift) by the liver enzyme, cytochrome P_{450}. This enzyme has been identified as the terminal oxidase involved in the metabolism of a large number of drugs and carcinogens by the hepatic microsomes. The mechanism of the transformation apparently involves the initial formation of a labile expoxide which then can undergo a rearrangement to the corresponding hydroxyl. Further evidence for the initial formation of a highly reactive epoxide along these lines has been published in a very provocative paper by Garner and his colleagues (45) at the McArdle Institute in Madison, Wisconsin. They found that if *Salmonella typhimurium* was incubated with aflatoxin B_1, rat liver microsomes, and a reduced NADPH generating system, a reduction occurred in the survival of the bacteria. Lethality appeared to depend on formation of a highly toxic metabolite of aflatoxin B_1 by a mixed function oxygenase system. The most toxic aflatoxins tested in this system possessed the double bond at the terminal furan, suggesting that the active metabolite may be an epoxide. Addition of RNA or DNA to the system inhibited the killing, and a covalently bound nucleic acid-aflatoxin derivative could be isolated. It has been shown that all chemical carcinogens that have been adequately examined covalently bind to nucleophilic target molecules such as DNA, RNA, or proteins. What role this plays in the carcinogenic process has yet to be elucidated.

Gurtoo and Dave (46) found evidence that an aflatoxin B_1 metabolite related to B_{2a} or its precursor was bound to rat liver RNA. The same year Swenson *et al.* (47) isolated a RNA–AfB_1 adduct that was obtained on incubation of rodent liver microsomes with aflatoxin B_1. Mild acid hydrolysis of the adduct gave 2,3-dihydroxy-AfB_1. It was again suggested that a AfB_1-2,3-epoxide is the probable precursor of the RNA–AfB_1 adduct. The reaction sequence postulated is shown in fig. 5.

In the calf, goat, sheep, pig, and rat, metabolism of aflatoxin B_1 to B_{2a} proceeds very poorly. The main route of attack is probably by formation of other

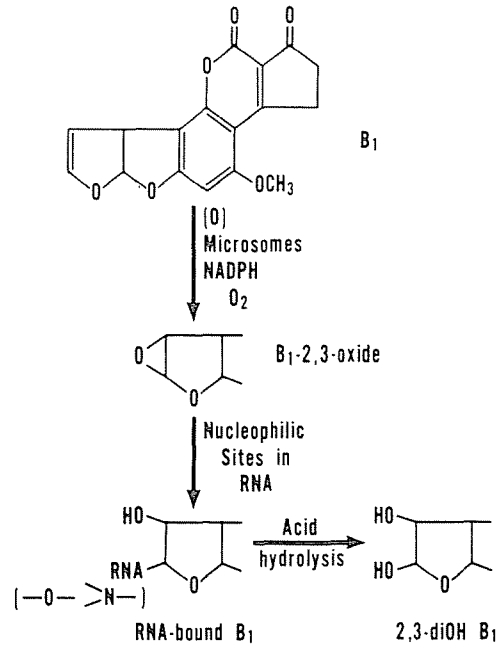


FIG. 5. *Probable mechanism of binding of aflatoxin B_1 to RNA by liver microsomes.* (ref. 47).

aflatoxin analogues via hydroxylation by the liver microsomes to give products such as aflatoxins M_1, P_1, and Q_1 (37, 48). The hydroxylase in rats resulting in M_1 formation has been shown to be inducible, and administration of aflatoxin or phenobarbital increases 4-hydroxylase activity 2.5 to 3.5 times (49). Hydroxylations, however, may not constitute a true detoxification; aflatoxin M_1 is as toxic and carcinogenic as B_1. Rather, the addition of a second alcohol group makes these compounds more water-soluble; hence they are free to form conjugates such as glucuronides and sulphates which are excreted in the urine. In monkeys and chickens, conjugates of M_1 and P_1 have been detected in the urine (50).

Attempts have been made to account for the varying effects and susceptibility of different hosts to B_1. Mature animals of a given species are generally more resistant than young ones, implying that aflatoxin-transforming enzymes develop in the liver with age. However, studies by Patterson and his colleagues (37) in England on avian and mammalian species have shown that there is no simple correlation between the ability of liver tissue, the principal site of metabolism, to metabolize aflatoxin and an animal's susceptibility to aflatoxin poisoning. In fact, duckling liver metabolizes aflatoxin very rapidly *in vitro,* although the species is sufficiently susceptible for day-old birds to be used widely in a sensitive bioassay for the toxin. In surveys using crude liver microsomal preparations, it has been shown that the overall rate of $NADPH_2$-dependent aflatoxin metabolism varied from only 0.3 nmoles/g tissue/min in the rat, a species highly susceptible to hepatomas, to 66 nmoles/g tissue/min in the duck under identical conditions (37). Thus, the untransformed toxin survives long enough in rat liver for it to be regarded as the molecular form causing tissue damage, whereas in species like the duck, a high rate of metabolism probably indicates that a metabolite is involved.

It is difficult in *in vitro* systems to quantify the relative importance of the soluble and microsomal pathways for aflatoxin metabolism because of the protein-binding properties of aflatoxin B_{2a} to form unstable adducts. This binding of B_{2a} could play a fundamental part in the acute toxic action of aflatoxin: for example, by the binding and inhibition of key enzymes of intermediary metabolism leading to hepatic cell necrosis. This hypothesis is not necessarily at variance with the observation that administered B_{2a} is nontoxic because, as stated earlier, it is possible that in its formation *in vivo,* a very toxic, short-lived expoxide may result initially from enzymic attack on the vinyl ether double bond of the terminal furan.

The active cytoplasmic reductive pathway in bird livers probably exerts a modifying effect on acute toxicity; but since the pathway is reversible, it may do no more than act as a reservoir for aflatoxin which is subsequently converted to B_{2a} or bound to intracellular structures that produce a chronic effect upon the liver.

The carcinogenic action of aflatoxin is also thought to depend upon its interaction with nucleic acids and, possibly, on its interference with membrane-polysome interaction by competing for sex-determined binding sites usually occupied by oestrone or testosterone (51). Aflatoxin and steroids compete for sites on the membrane that are responsible directly or indirectly for polysome binding (52). Animals, like the rat, that are capable only of slow aflatoxin metabolism would appear to be the most vulnerable to this kind of chronic liver damage by the untransformed toxin. However, in any species, the possibility of chronic liver damage by unchanged aflatoxin is real when dosing or feeding is prolonged. Thus hepatomas have been induced in the duck during a long-term feeding experiment, although acute effects of aflatoxicosis are usually associated with this species. In the money, 4.5 years are required to initiate hepatomas (53).

Animals that actively metabolize aflatoxin to B_{2a} seem to be particularly vulnerable to acute hepatotoxic effects, and survivors from the effect of a single

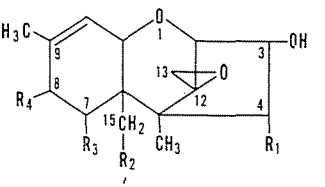
sub-lethal dose tend to escape chronic liver damage. However, those with an additional cytoplasmic reductive pathway are potentially liable to suffer protracted acute type and/or even chronic effects if this pathway is considered to be an aflatoxin reservoir as previously suggested.

One factor that has not been mentioned so far in this discussion is the transport of aflatoxin into the liver cell. This may prove to be a decisive consideration in the eventual understanding of the relationship between the toxicity of aflatoxin and its metabolism (37). For example, although mouse liver metabolizes aflatoxin much faster than rat liver, it is probably less susceptible to acute poisoning because the toxin is less efficiently taken up by mouse hepatocytes than by those of the rat.

In summary, once the toxin has entered the liver cell, the factors causing tissue injury in a particular animal species may be dictated by the rate and pattern of aflatoxin metabolism. When it is metabolized slowly, untransformed toxin is probably the active molecular species, with chronic liver damage the probable result. When it is metabolized rapidly, metabolites rather than the original toxin would seem to be involved.

TRICHOTHECENES

The 12,13-epoxy-Δ^9-trichothecenes have been implicated in a variety of mycotoxicoses involving on a large scale both humans and animals; these diseases include alimentary toxic aleukia, stachybotryotoxicosis, moldy corn toxicosis, and the refusal-vomition phenomenon. About 27 naturally occurring trichothecenes have been isolated to date, but fig. 6 shows only structures of those trichothecenes that are of current research interest.



	R_1	R_2	R_3	R_4
Nivalenol	OH	OH	OH	=O
Fusarenon-X	OAc	OH	OH	=O
T-2	OAc	OAc	H_2	$OCOCH_2CH(CH_3)_2$
Vomitoxin	H_2	OH	OH	=O

FIG. 6. *Structures of emetic trichothecenes.*

Alimentary toxic aleukia (ATA) in humans has been reported to occur primarily in Russia and results from the consumption of overwintered moldy cereals. A number of fungi have been implicated, but circumstantial evidence indicates that *Fusarium poae* and *F. sporoteichiodes* are probably the most important ones involved (1).

Disease symptoms have been described by Joffe (1) and include "fever; a hemorrhagic rash; bleeding from the nose, throat, and gums; necrotic angina; extreme leucopenia; agranulocytosis; sepsis; and exhaustion of the bone marrow." Mortality has been as high as 60%. The Russians reported that four mycotoxins were involved, but none of these have been detected by other investigators. Recent analysis of a sample of one of the Russian toxin preparations, poaefusarin, revealed the presence of 2.5% of a trichothecene, T–2 toxin (54); other trichothecenes present were neosolaniol (0.14%) and T–2 tetraol (0.6%). In addition, zearalenone or F–2 toxin (0.43%), a member of another class of mycotoxins, was also detected. The trichothecenes are capable of eliciting the

hemorrhagic symptoms associated with ATA and could well be the causative agents involved in this disease.

Stachybotryotoxicosis is a mycotoxicosis of horses, calves, sheep, and swine caused by the ingestion of feed contaminated with *Stachybotrys atra*. Symptoms are typical of those reported for most trichothecenes and include dermal necroses and hemorrhaging of the intestinal tract. Recently Eppley and Bailey (55) isolated five 12,13-epoxy-Δ^9-trichothecenes, including roridin E, from oats upon which *S. atra* had been grown; it is reasonable to believe that these are the compounds responsible for the disease.

Hsu *et al.* (56) in a fine example of scientific detective work demonstrated that T–2 toxin was associated with a lethal toxicosis in Wisconsin dairy cattle that had consumed corn molded primarily with *F. tricinctum*. The cows had extensive hemorrhaging on the serosal surface of all internal viscera typical of previously reported cases of moldy corn poisoning (57).

Reports of vomiting caused by consumption of moldy cereal grains, even when baked into bread in the form of flour, have been reported for animals and humans since 1916 by numerous investigators in various parts of the world. The causative agent eluded detection until 1973 when Vesonder *et al.* (58) isolated a new trichothecene, vomitoxin, from corn infected in the field with *F. graminearum*. These workers also speculated that vomitoxin was responsible for the refusal of this corn by swine. Vomitoxin does not appear to cause hemorrhaging and is less potent in causing dermal necrosis than is T–2 toxin. Its mode of action has been investigated.

TREMORGENS

Several mycotoxins produced by a variety of fungi are capable of causing sustained tremors in animals. Wilson and Wilson (59) isolated a tremorgenic toxin from *Aspergillus flavus,* but in yields too low to permit other than a determination of the molecular weight of 501 (60). Subsequently, Wilson and his colleagues (60) isolated a tremorgen-convulsant from two strains of *Penicillium crustosum* that had caused mycotoxicoses among sheep and horses. Soon after, a strain of *P. palitans* involved in the deaths of dairy cows in Illinois was isolated at our Laboratory (61). The toxin involved in the three outbreaks was a similar tremorgen that Dr. Wilson had named penitrem A and we, tremortin A; we have withdrawn our name in favor of Dr. Wilson's. We later isolated two additional closely related tremorgens that we now designate penitrem B and C (62).

Subsequently, two new tremorgenic substances were isolated from fungi not involved in field outbreaks (63, 64). None of the chemical structures of the tremorgens has yet been determined, although Cole and Kirksey (65) have presented evidence to support a 6-*O*-methylindole-type structure for verruculogen (a metabolite of *P. erruculosum*). Properties of the known tremorgens are shown in table 6.

TABLE 6. *Tremorgenic toxins from molds.*

Mold	Toxin	Source	M.W.	Elemental	m.p.	UV (mμ)	LD_{50}/kg mice (mg)
P. verruculosum	Verruculogen	Peanuts	551	$C_{30}H_{37}H_3O_7$	233–35	224,294	2.4
P. palitans	Penitrem A	Feedstuffs	633	$C_{37}H_{44}NO_6Cl$	237–39	233,295	1.1
P. cyclopium	Penitrem B		583	$C_{37}H_{45}NO_5$	185–95	227,286	5.8
P. crustosum	Penitrem C		—	—	—	—	—
A. fumigatus	Fumitremorgin A		579	$C_{33}H_{45}N_3O_6$	211–12	225,275, 295	<5
A. flavus	No name		501	—	—	—	—

Most laboratory animals and several farm animals appear to be susceptible to the neurotoxic effects of the tremorgens. All routes of administration are effective. The LD_{50} for mice is shown in table 6. At sublethal doses in mice, there is general irritability, ataxia, loss of grasping ability, and sustained tremors. At higher doses tremors are replaced by clonic or tetanic convulsions, with the mice passing into instant *rigor mortis* on death. Surviving mice appear to have suffered no ill effects. There was marked diuresis in some dosed mice and rats with a concomitant increase in total quantities of glucose and electrolytes in the urine (60). Hayes and Wilson (66), studying the effect of near lethal doses of penitrem A on brain and liver composition, noted that liver glycogen content was reduce 68% by 2 hr; liver protein, RNA, DNA, and lipids increased at 24 hr, 14%, 14%, 45%, and 178%, respectively; whereas total brain protein was reduced 38% by 6 hr.

In a pharmacological analysis of the tremors in mice induced by penitrem A, it was postulated that the toxin produces tremor by inhibiting the interneurons which inhibit the α-motor cells of the anterior horn of the spinal column (67). In this investigation tremor was inhibited by glycine, α-aminobutyric acid, diazepam, and mephenesin, all drugs capable of blocking the above-cited interneurons; anticholingeric drugs had no effect. Similar observations by Cysewski (68) on rabbits led him to believe that penitrem acted at the level of the spinal cord.

On another level, Wilson *et al.* (69) investigated tremorgenic effects on the neuromuscular junction of isolated rat phrenic nerve diaphram preparations. They found an increased frequency and mean amplitude in the miniature endplate potential which indicated that the toxin "stimulates or facilitates spontaneous release of transmitter packets." It was postulated that the tremorgen might act at pre- and post-junctional sites resulting in an increased release of transmitter packets and increased sensitivity of the post-junctional membrane.

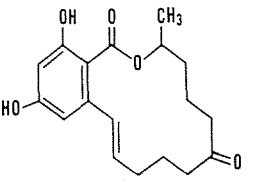
The clinical pathological changes in calves dosed with crude penitrem were reported by Cysewski (68) in a doctoral thesis. He noted elevations in plasma levels of pyruvic and lactic acid concomitant with marked tremors and suggested that the increases resulted from a shift to anaerobic glycolysis associated with marked muscular activity. There were also increases in plasma levels of creatine phosphokinase, lactic dehydrogenase and glutamic, oxalacetic and pyruvic transaminases, probably as a result of leakage from muscle during tremoring. Gross pathological changes were not noted and the only histological change was an increase of liver fat. The various changes observed were interpreted as a secondary effect of the toxicosis.

ZEARALENONE (F-2 TOXIN)

Various reports in the literature since 1928 indicate that estrogenism in swine is associated with consumption of moldy corn (70, 71). Subsequently, Stob *et al.* (72) isolated an anabolic uterotrophic compound from corn infected with *Gibberella zeae* (imperfect stage, *F. graminearum* or *F. roseum*) that appeared to be the cause of the estrogenic syndrome in swine. These researchers partially characterized the toxin, but later Urry *et al.* (73) determined the compound to be an enantiomorph of 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcyclic acid lactone which they gave the name zearalenone, the same substance called F-2 by Christensen *et al.* (74) and Microcha *et al.* (75) at the University of Minnesota (fig. 7). The estrogenic syndrome in swine primarily involves the genital system and in the prepuberal gilt is characterized by a swollen edematous vulva that may progress to vaginal prolapse; the uterus is also enlarged and endematous, the ovaries are shrunken and abortion can ensue. Young males can show feminizing effects such as atrophied testes and enlarged mammary glands (76).

This disease has been reported from a number of European countries and the United States. Symptoms are readily alleviated by replacement of moldy corn in the ration with sound corn.

Various derivatives of F–2 have been prepared that have greater anabolic activity than the parent compound. Thus, reduction of the ketone to a hydroxyl and reduction of the olefinic double bond results in a derivative that is currently being marketed as an anabolic agent. It has 3–4 times the activity of the parent substance and is ued to increase rate of gain and feed efficiency in



$C_{18}O_5H_{22}$

Structure of F-2 (Zearalenone)

FIG. 7. *Structure of F–2 (zearalenone).*

calves, feedlot steers, and lambs, apparently without estrogenic side effects if given properly. Although zearalenol does not have the molecular structure of a hormone, it does elicit a hormonal-like response in target animals. Its exact mode of action is uncertain but implanted animals show an increase in weight of the pituitary and adrenal glands, a level of somatotrophin 3.5 times higher than control animals, an increase in blood sugar and insulin production, and a decrease in blood urea nitrogen (77, 78). This suggests that zearalenol may cause the anabolic response by mediating the function of the pituitary gland. Whether this involves direct stimulation of this gland or of the hypothalmus which, in turn, produces release factors that affect pituitary action is not known.

CONCLUSIONS AND SUMMARY

Mycotoxins can be produced by a variety of fungi on food and feedstuffs that on consumption cause disease in both man and animals; symptoms can be either acute or chronic. Although acute symptoms are more dramatic, chronic effects may be more important in that they are insidious, difficult to detect, and can unknowingly cause considerable economic losses in livestock as a result of reduced feed efficiency and weight gains. Symptoms in mycotoxicoses tend to be nonspecific, hence difficult to diagnose. Many of the mycotoxins are hepatotoxins but other organs of the body can be and often are involved.

The problem is worldwide, not confined to any geographic area, and is extremely complex since mycotoxins can be produced on grains in the field, during harvest and processing, and during storage of any given food or feed. The tendency of these toxins to occur in comparatively low concentrations complicates detection and analyses; the problem may be further exacerbated by the potential occurrence of mixed toxins and of toxins bound to the substrate in which they may be produced, which render them difficult to detect.

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